

# PREPARATIVE LOADABILITY AS A FUNCTION OF pH

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## **OVERVIEW** -

Preparative chromatography is an important tool in the preparation of milligram to gram quantities of pure compound for further research such as structure elucidation and early toxicity studies. In this poster, we are demonstrating the rather dramatic effect of pH on the preparative loadability of ionizable compounds, both in theory and in practice. The practical examples show the increase in load, the reduction in column volume, and the use of the atcolumn dilution principle.

# LOADABILITY OF IONIZABLE COMPOUNDS -

As shown in the theory section, the preparative loadability of ionizable compounds such as amines and carboxylic acids depends strongly on the charge state of the compound. In practice, we have found roughly a 50-fold difference in loadability between the ionized form and the non-ionized form of the same compound. The examples shown here illustrate this effect. The columns used were XTerra® and XTerra®Prep columns that can be used at alkaline pH.

## **THEORY** -

The simplest description of the change in retention and peak shape with increasing sample load is the Langmuir sorption isotherm:

$$\frac{q}{q_{\infty}} = \frac{K \cdot c}{1 + K \cdot c}$$

q = surface concentration

q¥ = surface concentration at saturation

- c = concentration in the liquid
- K: constant defining the slope of the isotherm

For ionic compounds, the sorption is modified by the mutual repulsion of the adsorbed ions:

$$\boxed{\frac{q}{q_{\infty}} = \frac{K \cdot c \cdot f(q)}{1 + K \cdot c \cdot f(q)}}$$

q = surface concentration q¥ = surface concentration at saturation c = concentration in the liquid K: constant defining the slope of the isotherm

Mutual repulsion of the ions is expressed by the Ståhlberg theory:

$$f(q) = e^{-z^2 \cdot B \cdot q}$$
$$B = \frac{F^2}{\kappa \cdot \varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T} \cdot \frac{I_0(\kappa \cdot r)}{I_1(\kappa \cdot r)}$$



e = dielectric constant

I = Bessel functions

k = thickness of double layer

resulting in a modified isotherm:



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# **ADSORPTION ISOTHERMS -**



Table 1. Absorbtion Isotherm Table

## **PEAK FORM THEORY -**



Table 2. Peak Form Theory Graph

# LOADABILITY OF AN ACID -



Table 3. Loadability of an Acid

# LOADABILITY OF A BASE -



Table 4. Loadability of a Base

In agreement with theory, the loadability of both an acid (Table 3) and a base (Table 4) is superior in the non-ionized form. For acids this means that one should use an acidic pH and for bases a basic pH for optimum preparative chromatography.

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# LOADABILITY COMPARISON -Low pH High pH Total load 0.77 mg Total load 15.45 mg Total load 1.5 mg Total load 30.9 mg Total load 61.8 mg Total load 3.0 mg ΑU Total load 123.6 mg Total load 6.0 mg Total load 12.0 mg Total load 247.2 mg Total load 494.4 mg Total load 25.0 mg Time (min) Time (min)





Figure 1. Comparison of the isocratic preparative separation under acidic (top) and basic conditions (bottom) For this compound, the difference in loadability for equal peak shape was a **factor of 60!!!** 

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# **CURVE FIT RESULTS -**

to an empirical derivative of the charge-repulsion equation:

$$q = k_0 \cdot \phi \cdot \frac{c}{1 + D^* \cdot c \cdot e^{-B^* \cdot q}}$$

	<u>Oxacillin</u>	Cloxacillin	Dicloxacillin	Diphenhydramine	Oxybutynin	Terfenadine
ko	38.7	32.5	35.9	41.7	49.0	44.0
D [L/mol]	4718	4163	4565	2688	8037	40200
B [L/mol]	6.61	7.70	7.1	3.61	8.28	47.9
r <sup>2</sup>	0.999995	0.99999	8 0.999998	0.999996	0.999995	0.999997
%organic	7.2	9.9	13.5	6.3	15.3	27.9

# **STRUCTURES OF TEST ANALYTES -**



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# **CONCLUSION** -

The preparative loadability changes drastically with the ionization of a compound. The ionized form has between a 20- and 60-fold lower loadability than the un-ionized form of the same compound. Basic compounds should be chromatographed at basic pH and acidic compounds at acidic pH. This phenomenon has a significant impact on the cost of preparative chromatography. A reduction in column size leads to other savings as well, such as solvent consumption, instrument requirements and/or time.

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